CLAIMS 1-7 ARE NOT RENDERED OBVIOUS BY KIKUCHI ET AL. IN VIEW OF U.S. PATENT 6,413,774

The Examiner has rejected claims 1-7 under 35 U.S.C. §103(a) as allegedly being unpatentable over Kikuchi et al. in view of U.S. Patent 6,413,774. In support of this rejection, the Examiner contends that Kikuchi et al. teach separately digesting plus strand polynucleotides and minus strand polynucleotides with the endonuclease DNase I. Kikuchi et al. also allegedly teach combining the fragments and amplifying to generate a polynucleotide sequence encoding one or more protein motifs having altered characteristics. The Examiner acknowledges that Kikuchi et al. does not teach the use of an exonuclease, but contends that U.S. Patent 6,413,774 teaches the use of an exonuclease for "removing non-templated nucleotides."

Applicants respectfully disagree with the Examiner's position. The criterion for determining obviousness under §103 is whether the prior art supplies some motivation or incentive to one of ordinary skill in the art to arrive at the invention as claimed. In re Dow Chemical Company, 5 U.S.P.Q. 2d 1929 (Fed. Cir. 1988). Obviousness cannot be established by combining teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. In re Fine, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988). Moreover, the teaching or suggestion supporting the desirability of the combination must be found in the prior art, not in applicant's disclosure. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992). Under these standards, Applicants submit that neither of the cited references, considered singly or in combination, render the claimed invention obvious.

Specifically, Applicants submit that Kikuchi et al. provide no motivation whatsoever to replace the DNase I digestion step with an exonuclease digestion step because no mention is made in the paper of any problems or disadvantages

associated with the use of DNase I. Upon reading Kikuchi et al., a skilled person would be motivated to use the same nuclease because DNase I is presented as having advantageous properties. Indeed, Kikuchi et al. note at page 133 that they "took advantage of DNase I to cleave ssDNA." Hence, Kikuchi et al. fail to provide motivation for a skilled person to replace DNase I in the described methods with another nuclease.

Additionally, Applicants submit that a person skilled in the art, upon reading U.S. Patent 6,413,774, would not be motivated to combine the teachings of U.S. Patent 6,413,774 with those of Kikuchi et al. U.S. Patent 6,413,774 discloses several methods for generating variant polynucleotides, both *in vitro* and *in vivo*. A common feature of the *in vitro* methods is that they comprise a step of generating a population of single-stranded fragments from a parent polynucleotide, or "template polynucleotide." Nuclease digestion is identified as one of several methods by which the fragments may be generated (see column 6, lines 10 to 12). Once generated, the single-stranded fragments are then recombined to generate a library of variant polynucleotides.

Significantly, U.S. Patent 6,413,774 teaches that the single-stranded fragments should be generated by first fragmenting a double-stranded parent polynucleotide and subsequently denaturing the double-stranded fragments into single-stranded fragments (see, for example, column 6, line 66 to column 7, line 61). At column 23, lines 13-18, U.S. Patent 6,413,774 also notes that:

> The template polynucleotide often should be double-stranded. A <u>double stranded nucleic acid molecule is required</u> to ensure that all regions of the resulting single-stranded nucleic acid fragments are complementary to each other and thus can hybridize to form a double-stranded molecule. [emphasis added]

Furthermore, U.S. Patent 6,413,774 specifically suggests employing DNase I for the fragmentation of polynucleotides (see, for example, column 23, lines 39-40 and column 28, lines 1-4). Indeed, in all the examples of *in vitro* shuffling methods described in U.S. Patent 6,413,774, nuclease digestion is performed on **double stranded** parent polynucleotides with **DNase I** (see Examples 1-5, 7, 13-16, and 18-20).

Thus, the invention of U.S. Patent 6,413,774 comprises a step of fragmenting double-stranded parent polynucleotides, exemplified by treating the polynucleotides with the endonuclease DNase I, and denaturing the resultant double-stranded fragments to generate single-stranded fragments. However, U.S. Patent 6,413,774 fails to disclose or suggest the nuclease digestion of single-stranded parent (i.e. template) polynucleotides. In contrast, Kikuchi et al. describe a method involving the digestion of single-stranded polynucleotides, which overcomes problems associated with the digestion of double-stranded polynucleotides. Hence, Applicants submit that 1) a person skilled in the art would not be motivated to combine the teaching of a method comprising the generation of double-stranded fragments with the teaching of a method entailing the digestion of single-stranded polynucleotides; and 2) even if the skilled artisan would attempt to combine the teachings of Kikuchi et al. and U.S. Patent 6,413,774, then the skilled artisan would be left with the overwhelming indication from both teachings that DNase I is a superior nuclease and would not be motivated to test or employ other nucleases such as the exonucleases of the instantly claimed invention.

The Examiner asserts that U.S. Patent 6,413,774 teaches employing exonucleases in the described method for DNA shuffling. Thus, according to the Examiner's reasoning, it would be obvious to a person skilled in the art to modify the

method of Kikuchi et al. by replacing the endonuclease DNase I with an exonuclease described in U.S. Patent 6,413,774. In support of this assertion, the Examiner cites column 49, line 65 to column 50, line 12 of U.S. Patent 6,413,774.

However, a closer inspection of U.S. Patent 6,413,774 reveals that the Examiner's reasoning is flawed. Indeed, the disclosure at column 49, line 65 to column 50, line 12 does not teach the use of an exonuclease in the fragmentation of the double-stranded parent polynucleotides. Rather, this passage teaches that an exonuclease activity may be **added** to the shuffling method in order to remove "non-templated nucleotides introduced at 3' ends of product polynucleotides in shuffling amplification reaction catalyzed by a non-proofreading polymerase." Thus, the exonuclease activity is not described as generating the polynucleotide fragments of the method, but rather is taught to be useful after fragmentation has occurred during the reannealing and amplification stage. Indeed, a similar disclosure can be found at column 9, lines 35 to 56 wherein the desired exonuclease activity is described to be present in Pfu polymerase and other similar polymerases, which obviously can not be employed to generate nucleic acid fragments in the same manner as the instantly claimed exonucleases. Therefore, Applicants submit that a skilled artisan apprised of the teachings of U.S. Patent 6,413,774 would only consider adding an exonuclease activity during the amplification step in order to remove errors generated by a non-proofreading polymerase. U.S. Patent 6,413,774, however, clearly neither discloses nor suggests the use of an exonuclease to fragment the parent polynucleotides and, accordingly, fails to motivate a skilled person from employing an exonuclease in place of DNase I in the method of Kikuchi et al.

As further support for the non-obviousness of the methods of the present invention over Kikuchi et al., Applicants respectfully refer the Examiner to the evidence

submitted on May 19, 2003 demonstrating the unexpected advantage obtained when using an exonuclease to digest the parent DNA. Specifically, Applicants refer to Appendix 2 of Dr. Uhlen's declaration which demonstrates the improved control of fragment size by exonuclease digestion as opposed to endonuclease digestion. This control in fragment size, and not the ability of the exonucleases to excise non-templated nucleotides introduced during polymerase amplification, permits greater control of the variant polynucleotides produced by the methods of the instant invention. Such an advantageous effect is neither disclosed in nor suggested by the prior art documents cited by the Examiner.

In light of all of the foregoing, Applicants submit that rejection of claims 1-7 under 35 U.S.C. §103(a) as allegedly being unpatentable over Kikuchi et al. in view of U.S. Patent 6,413,774 is untenable and therefore, respectfully request its withdrawal.

CONCLUSION

In view of the amendments presented herewith, and the foregoing remarks, it is respectfully urged that the rejections set forth in the January 27, 2004 Official Action be withdrawn and that this application be passed to issue.

In the event the Examiner is not persuaded as to the allowability of any claim, and it appears that any outstanding issues may be resolved through a telephone interview, the Examiner is requested to telephone the undersigned attorney at the phone number give below.

Respectfully submitted,
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